PATENT CLAIMS

(1) A method for determining the concentration of thrombin inhibitors in a non-turbid body liquid or a non-turbid extract from a body liquid, comprising the following steps:

a) the body liquid is taken from a living body, and the body liquid is subjected to a separation from the turbid matter, if necessary,

b) to the non-turbid body liquid obtained in step a) are added a coagulation-inhibiting substance not interfering in the transformation prothrombin/active meizothrombin or Mtdesfgl, resp., a chromogenic or fluorogenic substrate not dissociable by active meizothrombin or Mtdesfgl, resp., and a substance dissociating prothrombin into meizothrombin or Mtdesfgl, resp., and as an option prothrombin,

c) the solution or mixture, resp., obtained in step b) is subjected to a wavelength-selective light absorption or light emission measurement as a function of the time,

d) from the reduction of the light absorption or light emission in step c) per time unit is determined the amount of the thrombin inhibitor included in the body liquid by comparison to previously determined standard curves.

(2) A method for determining the activity of thrombin inhibitors in a non-turbid aqueous liquid, comprising the following steps:

a) a body liquid is taken from a living body, and the body liquid is subjected to a separation from the turbid matter, if necessary, or a non-turbid liquid is synthetically produced,

b) to the non-turbid body liquid obtained in step a) are added a given amount of thrombin inhibitor, if applicable a coagulation-inhibiting substance not interfering in the transformation prothrombin/active meizothrombin or Mtdesfgl, resp., a chromogenic or fluorogenic substrate not dissociable by active meizothrombin or Mtdesfgl, resp., and a substance dissociating prothrombin into meizothrombin or Mtdesfgl, resp., or meizothrombin or Mtdesfgl, resp., and as an option prothrombin,

c) the solution or mixture, resp., obtained in step b) is subjected to a wavelength-selective light absorption or light emission measurement as a function of the time,

d) from the reduction of the light absorption or light emission in step c) per time unit is determined the activity of the thrombin inhibitor by comparison to previously determined standard curves.

3. A method according to claim 1 or 2, wherein the coagulation inhibiting substance not interfering in the transformation prothrombin/active meizothrombin or Mtdesfgl, resp., is selected from the group "calcium-complex forming agents, heparin, heparinoids, anti-thrombin III, protein C, fibrin polymerization inhibiting substances and mixtures of such substances".

4. A method according to one of claims 1 to 3, wherein the substance dissociating prothrombin into meizothrombin or Mtdesfgl, resp., is selected from the group of the snake venoms or snake venom fractions.

5. A method according to one of claims 1 to 4, wherein the substance dissociating prothrombin into meizothrombin or Mtdesfgl, resp., is ecarin.

6. A method according to one of claims 1 to 5, wherein the chromogenic substrate dissociable by active meizothrombin or Mtdesfgl, resp., releases p-nitroanilin under dissociation, and the light absorption measurement is performed at 405 nm.

7. A method according to one of claims 1 to 6, wherein in step c) a first absorption or emission measurement after 0 - 100 s, preferably 0 - 50, most preferably 5 - 15 s, and a second one after another 10 - 1,000 s, preferably 50 - 500s, most preferably 150 - 300 s, measured from the addition of the substance dissociating prothrombin into meizothrombin or Mtdesfgl, resp., are performed.

8. A method according to one of claims 1 to 7, wherein the thrombin inhibitor is hirudin, a hirulog or a synthetic thrombin inhibitor.

9. A test kit for determining the concentration of thrombin inhibitors in a non-turbid body liquid or a non-turbid extract from a body liquid, comprising the following kit components: K1) a solution of a coagulation-inhibiting substance not interfering in the transformation prothrombin/active meizothrombin or Mtdesfgl, resp., K2) a chromogenic or fluorogenic substrate dissociable by active meizothrombin or Mtdesfgl, resp., and K3) a solution of a substance dissociating prothrombin into meizothrombin or Mtdesfgl, resp., wherein component K3) may be replaced or complemented by a component K3a) of a solution with meizothrombin or Mtdesfgl, resp.

10. A test kit for determining the activity of thrombin inhibitors in a non-turbid body or in a non-turbid extract from a body liquid or in a non-turbid non-natural aqueous liquid, comprising the following kit components as an op-

tion K1) a solution of a coagulation-inhibiting substance not interfering in the transformation prothrombin/active meizothrombin or Mtdesfg1, resp., K2) a chromogenic or fluorogenic substrate dissociable by active meizothrombin or Mtdesfgl, resp., and K3) a solution of a substance dissociating prothrombin into meizothrombin or Mtdesfgl, resp., wherein component K3) may be replaced or complemented by a component K3a) of a solution with meizothrombin or Mtdesfgl, resp.

11. A test kit according to claim 9 or 10, wherein the kit components are separated from each other but provided in a single test kit package.

12. A test kit according to claim 9 or 10, wherein as an optional additional kit component, a solution with prothrombin is provided.

13. Thrombin inhibitors, which are available by the following steps:

A) elements of a group of prospective thrombin inhibitors are submitted subsequently or separately and simultaneously in a given and preferably identical concentration to a method according to one of claims 2 to 8,

B) the reduction of the light absorption or light emission per time unit is determined for each prospective thrombin inhibitor and compared to the light absorption or light emission per time unit of a given, preferably identical concentration of hirudin determined under identical conditions,

C) those prospective thrombin inhibitors are selected the reduction of the light absorption or light emission of which per time unit corresponds to at least 10 % of the corresponding reduction when hirudin is used.

Fig. 1

Hirudin concentration (µg/ml plasma)